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Survival and Growth of 'Tanytarsus dissimilis' (Chironomidae) Exposed to Copper, Cadmium, Zinc, and Lead

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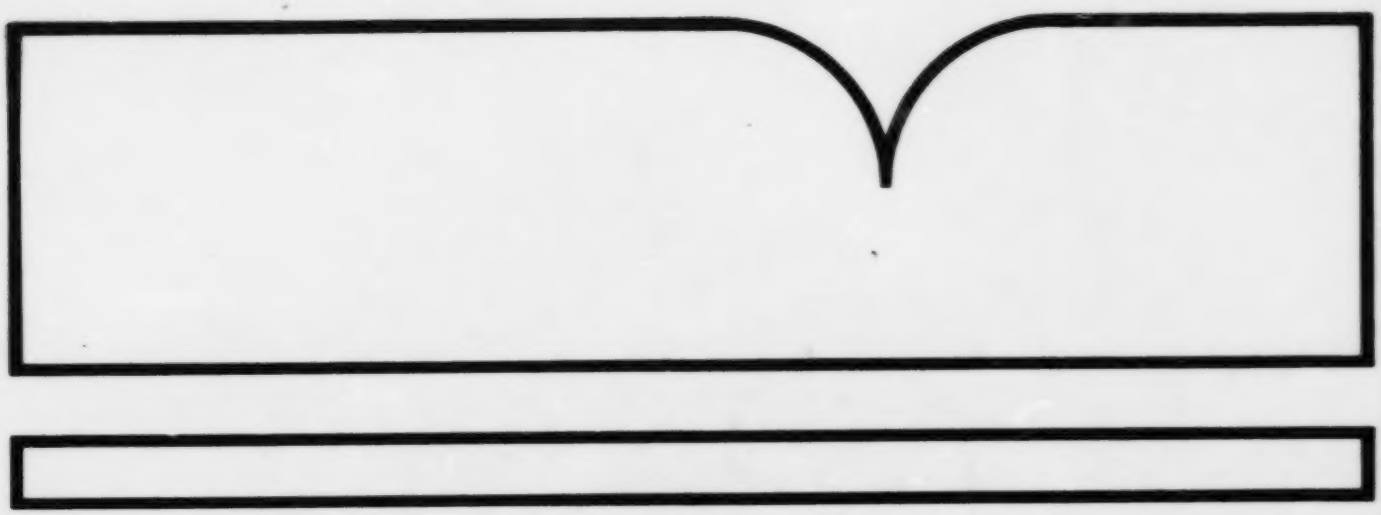

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16. ABSTRACT

Tanytarsus dissimilis (Johannsen) was exposed to four heavy metals. Static exposure began during embryogenesis and continued through hatching and larval development to the 2nd or 3rd instar. The LC50 concentrations for cadmium, copper, and zinc were 3.8, 16.3, and 36.8 µg/l, respectively. The LC50 for lead was 258 µg/l. Growth was not reduced at exposure concentrations less than the LC50.

The LC50 concentrations obtained in this insect exposure are as much as 1,600 times lower than other insect exposures reported in the literature. This is probably due to a combination of exposure of this insect during important life cycle events and species-specificity.

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Survival and Growth of *Tanytarsus dissimilis* (Chironomidae) Exposed to Copper, Cadmium, Zinc, and Lead

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Abstract. *Tanytarsus dissimilis* (Johannsen) was exposed to four heavy metals. Static exposure began during embryogenesis and continued through hatching and larval development to the 2nd or 3rd instar. The LC50 concentrations for cadmium, copper, and zinc were 3.8, 16.3, and 36.8 μg/l, respectively. The LC50 for lead was 258 μg/l. Growth was not reduced at exposure concentrations less than the LC50.

The LC50 concentrations obtained in this insect exposure are as much as 1,600 times lower than other insect exposures reported in the literature. This is probably due to a combination of exposure of this insect during important life cycle events and species-specificity.

Literature contains only a few papers on the effects of heavy metal pollution on aquatic insects. Several field reports show that certain insect species are resistant to high heavy-metal concentrations. The chironomid *Cricotopus bicinctus* was the dominant benthic invertebrate in a stream which contained copper and chromium at concentrations of 2.2 and 2.5 ppm, respectively (Surber 1959). Cherry *et al.* (1979) showed that the most successful invertebrates in an area dominated by a coal ash and thermal discharge were odonates and chironomids. The chironomids were found in areas which had average water concentrations of 120 μg/l cadmium, 400 μg/l copper, and 370 μg/l zinc. Reports from laboratory exposures generally support the field studies. For example, the stonefly, *Acroneuria lycorias*, the mayfly, *Ephemerella subvaria*, and the caddisfly, *Hydropsyche betteni* were exposed for 4 to 14 days to nine heavy metals (Warnick and Bell 1969) and the LC50 concentration ranged from 320 μg/l for a four-day exposure of a mayfly to copper to 64,000 μg/l for a 14-day exposure of the stonefly to lead. Other reports (Rehwoldt *et al.* 1973; Thorp and Lake 1974; and Clubb *et al.* 1975) have supported the earlier study, which show that other aquatic insects are also resistant to heavy metals in the water.

Two recent reports have indicated that the apparent resistance of aquatic insects to heavy metal pollution may not be a general response. The stonefly (*Pteronarcys californicia*) and the mayfly (*Ephemerella grandis*) were exposed

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for 14 days to copper, lead, silver, or zinc (Nehring 1976). The LC50 concentrations for stoneflies exposed to lead, zinc and copper were greater than 10,100 μg/l which supported the other studies. However, the LC50 for mayflies exposed to copper was 180 μg/l. The LC50 for silver was less than 1 μg/l for the mayfly and 4 to 9 μg/l for the stonefly. The sensitivity of the two species to silver and the mayfly to copper indicates that at least some insects are not equally tolerant of heavy metal pollution. In the second report (Spehar *et al.* 1978) stoneflies, mayflies, caddisflies, amphipods and snails were exposed to cadmium and lead. The exposures of stoneflies to cadmium and caddisflies, snails, and stoneflies to lead showed an insensitivity to these metals. However, mayfly (*Ephemerella sp.*) and snail (*Physa integra*) cadmium LC50 concentrations were similar to those which show chronic exposure effects on fish (3 to 8 μg/l). A similar result was found for the amphipod (*Gammarus pseudolimnaeus*) exposure to lead (Spehar *et al.* 1978).

Although the toxicity-tests reported in the literature show a species-specific response, a fundamental deficiency is that the insects are usually in an intramolt status during the test. The total effect of a toxicant exposure during life cycle events such as embryogenesis, hatching, and molting have not been studied.

The objectives of this study were to determine the impact of copper, zinc, lead, and cadmium on the survival of the parthenogenetic chironomid *Tanytarsus dissimilis* Johannsen (1937) when exposed through several life cycle events. This insect was chosen because of its short life-cycle and ease in rearing. In the toxicity tests, the insects were continually exposed during embryogenesis, hatching, and larval development into the 2nd or 3rd instar. The total exposure time was 10 days, which is equal to about two-thirds of the life cycle at 22°C.

Materials and Methods

Source and Food

Tanytarsus dissimilis eggs used to initiate these experiments were obtained from a colony maintained at the Environmental Research Laboratory-Duluth for the last seven years. The food used for rearing was also used in the toxicity tests. It was prepared by adding 0.5 g of powdered dried grass (Cerophyl®) and 10 g of enriched trout-fry granules[1] to 250 ml of Lake Superior water and mixing vigorously in a blender for five min. The suspension was strained through a 250-mesh screen. The blender was rinsed with an additional 50 ml of lake water, which was added to the suspension. The food was refrigerated and mixed thoroughly each time an aliquot was removed (Biesinger and Christensen 1972). Food (1 ml/l) was added to the test solution at the beginning of each test. No additional feeding was necessary. Unfiltered Lake Superior water was used for all rearing and testing. The chemical characteristics of Lake Superior water, including the ambient heavy metal content was reported by Biesinger and Christensen (1972).

Test Method

For each experimental and control condition, 9 to 11 eggs less than 16 hr old were placed in each of four beakers containing 200 ml of test water. Hatching occurred in 2 to 3 days. Five concentrations

[1] Glencoe Mills Inc., Glencoe, MN 55336

Table 1. Chemical characteristics of the test water

Stem	Average ± 1 S.D.	n
Dissolved oxygen	8.7 ± 1.4[a]	6
pH	7.5 (7.3-7.7)[b]	32
Hardness as $CaCO_3$	46.8 ± 2.1	34
Alkalinity as $CaCO_3$	43.9 ± 2.1	28
Acidity as $CaCO_3$	2.6 ± 0.4	22
Copper	1.8 ± 0.96	3
Cadmium	<0.3	4
Lead	<5.0	4
Zinc	5.1 ± 3.2	6

[a] Mg/L except for pH and metals. Metals in μg/L
[b] Median and range

and a control were used in each test, which resulted in a total of 24 beakers per test. After addition of the eggs, each beaker was covered with a screen and placed in a shallow water bath to minimize evaporation. At the end of the 10-day exposure, the surviving larvae in each beaker were counted, removed and killed by submersion in boiling water. Length or head-capsule width were measured with a microscope equipped with a calibrated ocular micrometer.

Test beakers (400 ml) were prepared by washing with a detergent and water, rinsing with tap water, and placing in a 20% nitric acid solution for a minimum of two hr. Each beaker was then rinsed with tap water, distilled water, and finally with Lake Superior (=test) water. Control and test solutions were prepared 24 hr before the eggs were added.

A combination of Duro-Test (Optima FS) and wide spectrum Grow-Lux fluorescent bulbs provided a light intensity of 160 to 215 lux at the table top. An automatically controlled photoperiod of 16 hr was used in all tests. Temperature was 22° ± 1°C.

Chemical Measurements

Dissolved oxygen, acidity, alkalinity, pH, and hardness were determined at the beginning and end of the test with methods outlined by the American Public Health Association (1971). The results of these measurements and the copper, cadmium, lead, and zinc concentrations in the control water are shown in Table 1.

Metal stock solutions of $CdCl_2$, $CuCl_2$, $Pb(NO_3)$, and $ZnCl_2$ were prepared from reagent-grade chemicals with distilled water. Each concentration was prepared in a one L volumetric flask by adding measured amounts of stock solution along with one ml of food suspension. This was mixed and 200 ml dispensed to each of four test beakers used for each concentration. The remaining 200 ml was quantitatively analyzed for the specific metal.

Samples for metal analysis were taken before and after the completion of a test. All samples were acidified in their original containers with 0.1 ml of 1:1 HNO_3 per 100 ml of sample and allowed to stand for 24 hr. This procedure results in measured values which include the ambient concentration plus the additions due to the test water and food. Standard curves were prepared for each test before and after each sample set. To determine the concentration in the test water, two aliquots were analyzed from each 200 ml sample. Average peak height of each aliquot was compared by computer to the standard curve from which test solution concentrations were calculated.

The methods of analysis were verified by adding known amounts of each metal to the control water. Recovery was greater than 90% for Cu, Cd, and Zn. The recovery was 80% for lead.

Zinc concentrations were determined by atomic absorption with an air-acetylene flame. The detection limit for this method under the conditions of the experiment was 3 μg/l.

Cadmium, copper, and lead concentrations were determined on an atomic absorption spectrophotometer equipped with a graphite furnace.

The approximate detection limits for the analyses were: cadmium, 0.3 μg/l; copper, 1.0 μg/l; and lead, 0.2 μg/l.

Table 2. Cadmium, copper, lead, and zinc toxic concentrations for the chironomid *Tanytarsus dissimilis*

Metal	Number of tests	Average LC50	
		X ± S.D. μg/L	*M*
Cadmium	3	3.8 ± 3.5	0.03×10^{-6}
Copper	3	16.3 ± 3.4	0.26×10^{-6}
Lead	2	258	1.24×10^{-6}
Zinc	3	36.8 ± 14.6	0.56×10^{-6}

Statistical Analysis

The bioassays for each metal were repeated three times except for lead, which was done twice. The effect of the metal on survival was determined by calculating an LC50 for each test by using a straight line graphical technique (American Public Health Association 1971).

Results

The results of the tests are shown in Table 2. Three tests were completed with cadmium; the pooled average LC50 was 3.8 μg/l. The range of LC50's was from 0.4 to 7.5 μg/l. The pooled average LC50 for three copper tests was 16.3 μg/l with a range of 13.3-20.0 μg/l. Three tests were done with zinc. The range of LC50 concentrations for the zinc tests were 26.0 to 53.5 μg/l with an average of 36.8 μg/l. Lead was much less toxic than cadmium, copper, or zinc. The average LC50 for the two tests was 258 μg/l.

The LC50's of the metals expressed in terms of molarity are as follows: Cd, 0.03×10^{-6}M; Cu, 0.26×10^{-6}M; Zn, $0.56 \times 10^{-6}M$; and Pb, $1.24 \times 10^{-6}M$. Using these values, cadmium was 41, copper 5, and zinc 2 times as toxic as Pb which was the least toxic metal tested.

The cadmium and copper tests included concentrations that killed all the larvae. Copper concentrations above 28 μg/l and cadmium concentrations as low as 8 μg/l resulted in 100% mortality. Zinc was not tested at concentrations that were lethal to all the larvae. However, survival was only 10% at zinc concentrations of 80-100 μg/l. The concentration necessary for 100% mortality with lead was not determined.

Growth

The effect of the four heavy metals is shown in Figures 1, 2, and 3. Each line represents the results of a single toxicity test. The numbers on each line represent the number of larvae measured at that concentration. The numbers do not exactly represent the total survival at a concentration since, after being counted, some larvae were lost or destroyed during the collection and measuring process. The control larvae were, in all cases, in the 2nd or 3rd instar at the termination date.

The Figures show that until the LC50 concentration is exceeded, there is no indication of a growth inhibition. At concentrations greater than the average

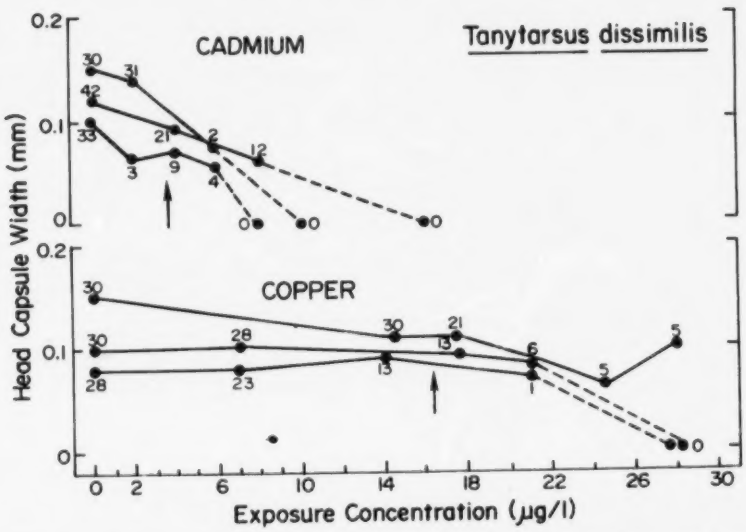

Fig. 1. Effects of cadmium and copper on growth and survival of *Tanytarsus dissimilis*. Each number represents the number of larvae measured at each concentration (●). The vertical arrows show the average LC50

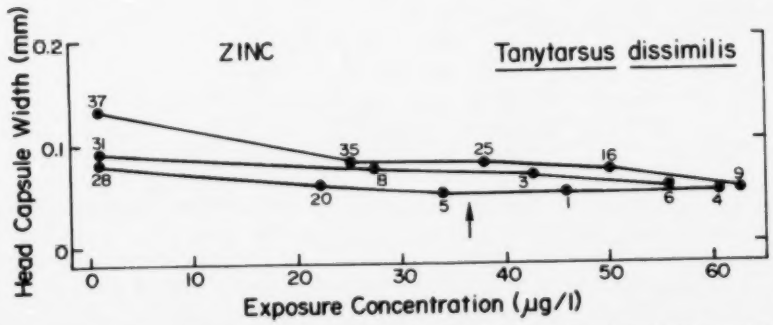

Fig. 2. Effect of zinc on growth and survival of *Tanytarsus dissimilis*. Each number represents the number of larvae measured at each concentration (●). The vertical arrow shows the average LC50

LC50, there is an indication of decreased growth with cadmium and zinc, however, the toxic effects had reduced the survival to 20% or less.

Discussion

A comparison of the results of these exposures with the reported values of others for aquatic insects shows that this insect is, in general, substantially more sensitive to the four metals studied. The results of these exposures for

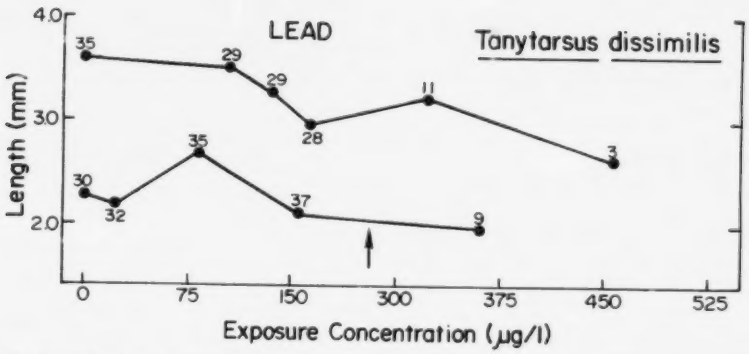

Fig. 3. Effect of lead on growth and survival of *Tanytarsus dissimilis*. Each number represents the number of larvae measured at each concentration (●). The vertical arrow shows the average LC50

cadmium provided an average LC50 value of 3.8 µg/l. This can be compared to LC50 values which range from a similar concentration of 3 µg/l for the mayfly *Ephemerella sp.* (Spehar *et al.* 1978) to >64,000 µg/l for *Hydropsyche sp.* (Warnick and Bell 1969). Similar comparisons can be made for the other metals used in this study, although in no other case is the comparison between our data and those of others, at least at the lowest comparison concentration, as close as with cadmium. In general, the LC50 concentrations reported here are 2 to 870 times lower for copper, 13 to 250 times lower for lead, and 43 to 1,600 times lower for zinc than other reported values in the literature for other aquatic insects (Warnick and Bell 1969; Nehring 1976; and Rehwoldt *et al.* 1973).

Interpretation and comparison of the results of one series of laboratory bioassays to other data rests on an understanding of the biology of the test animal and the condition of the test. The usual approach for determining the toxicity of heavy metals to benthic insects has been to collect the specimens from natural streams and to expose the animals for four days (Warnick and Bell 1969; and Rehwoldt *et al.* 1973). A few studies have exposed the insects for 14 to 28 days (Nehring 1976; and Spehar *et al.* 1978). Both of these approaches usually expose the animal during a specific stage and do not include developmental events. The insect used in this test will complete its life cycle in about 14 to 19 days at temperatures of 21 to 28°C (Nebeker 1973). Although the egg to egg life cycle is only 14 to 19 days, a 10-day test period was used because it allowed exposure through three life cycle events, embryogenesis, hatching, and molting. Also, enough food could be added at the beginning of the test to allow development into the 2nd or 3rd instar. The addition of food necessary to complete development in this static system would influence the metal-water complexes and cloud the results and interpretation.

The importance of exposure during sensitive stages has been reported by Clubb *et al.* (1975) for aquatic insects exposed to cadmium. Insects collected before November were more sensitive than those collected after November. They also reported that the incidence of molting was reduced in *Pteronarcys californica* and *Arcynopteryx signata*.

The results of these tests are not directly comparable to those which do not

expose through critical life cycle events. However, they do indicate that this insect is much more sensitive to specific heavy metals than the others tested and points toward the necessity of testing more than one species and including more than one life stage in a toxicity test.

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